Role of the Microbiologist in Infection Control and Hospital Epidemiology

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Disclosures
Objectives

- Understand the importance of the microbiology laboratory to infection control, the hospital epidemiologist, and the infectious disease physician.
- Understand the various techniques available to assist in an epidemiological investigation.

Infectious Disease Diagnostics

- Diagnostic tests for infectious diseases have changed to:
  - Detection of infectious agents/molecules/genes replacing growth and identification procedures.
  - Turn around time minutes to hours replacing days to weeks
  - Evolved through transitional research.
  - Direct impact patient care
  - Need hospital based studies to validate their clinical effectiveness.

Microbiology is now part of the healthcare team
Infection Prevention Programs

- Role in infections
  - Monitor
    - Understanding the epidemiology of HAIs
    - Determine rates of infections
    - Surveillance
  - Prevent
    - Intervene to prevent infections
    - Education
  - Control
    - Outbreak investigation
- The clinical microbiology laboratory is essential to a comprehensive infection prevention program

Role of the Microbiology Laboratory in Infection Control

- Specimen Collection
- Accurate Identification and Susceptibility Testing
- Laboratory Information Systems
- Rapid Diagnostic Testing
- Reporting of Laboratory Data
- Outbreak Recognition and Investigations-Molecular Typing
- Organism Storage
- Cultures of Specimens from Hospital Personnel and the Environment
Specimen Collection

- Educate on proper specimen collection and transport
  - Sputum versus spit (oropharyngeal flora)
- Monitor specimen quality
  - Sputum gram stains
- Reject improper specimens
  - Sputums with > 25 squamous epithelial cells, low PMNs, oropharyngeal flora

Accurate Identification of Healthcare-Associated Pathogens

Identify causative organisms rapidly and accurately to species level.

- Diagnostic
- Surveillance
- Environment

Expanding spectrum of organisms that colonize and infect seriously ill patients challenges the ability to identify and characterize pathogens accurately.
Healthcare-Associated Infections: Changing Microbiology

Mid-1990’s
- Decline in Enterobacteriaceae
- Increase in gram-positive cocci
- Emergence of fungi
- Recognition of viruses

2000 and beyond
- ESKAPE organisms
- Increase in resistant GNRs
  - ESBLs
  - Carbapenem-resistant Enterobacteriaceae (CRE)
  - Carbapenem-resistant Acinetobacter
  - Carbapenem R Pseudomonas aeruginosa
- VISA, VRSA
- Emerging pathogens
  - SARS
  - Monkeypox
  - Norovirus
  - MERS
- Fungi
  - Amphotericin R moulds
  - Fluconazole resistant yeasts

Major Pathogens of HAIs

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. (%) of pathogens</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>12,635 (15.6)</td>
<td>1</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>9,351 (11.5)</td>
<td>2</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>9,261 (11.4)</td>
<td>3</td>
</tr>
<tr>
<td>Klebsiella (pneumoniae/oxytoca)</td>
<td>6,470 (8.0)</td>
<td>4</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>6,111 (7.5)</td>
<td>5</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>5,484 (6.8)</td>
<td>6</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>4,275 (5.3)</td>
<td>7</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>3,821 (4.7)</td>
<td>8</td>
</tr>
<tr>
<td>Other Candida spp. or NOS</td>
<td>3,408 (4.2)</td>
<td>9</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>3,314 (4.1)</td>
<td>10</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>2,409 (3.0)</td>
<td>11</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>2,031 (2.5)</td>
<td>12</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>1,737 (2.1)</td>
<td>13</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>1,990 (1.8)</td>
<td>14</td>
</tr>
<tr>
<td>Other*</td>
<td>9,304 (11.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>81,139 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Over a 3rd of HAIs are Gram-negative pathogens

Data from CDC 2009-2010
Sievert et al. ICHE 2013
Accurate Identification of Pathogens

Identify causative organisms rapidly and accurately to species level.

- Educated and well trained personnel
- Automated systems
  - Know the limitations of each system
  - Good for identification of aerobic gram positive and gram-negative bacteria, not good for many nonfermentative gram-negatives
- Molecular testing
  - Live versus Dead
  - Cross-reactivity

Matrix Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF)

- Measures particles based on their mass to charge ratio
Susceptibility Testing of Healthcare-Associated Pathogens

Perform accurate susceptibility testing

- Implementation of multiple techniques
  - Rapid automated systems
  - Disk Diffusion
  - E-test
- Survey for MDR organisms
- Detect unexpected antimicrobial resistance

Antibiotic Susceptibility Testing

- Disk Diffusion
- Dilution
- Dilution and Diffusion

- Kirby Bauer
- Tube Dilution
- Agar Dilution
- E test

Qualitative

Quantitative
**Klebsiella pneumoniae**

- Susceptible
- Resistant to all

**Minimal Inhibitory Concentration (MIC)**
- The least amount of antimicrobial agent, usually in $\mu$g/ml, that prevents the growth of the organism in vitro.

**Low to High Antibiotic Concentrations**
Dilution and Diffusion-Epsilometer test (E-test)

Predefined stable gradient of 15 antibiotics concentrations impregnated on the strip

MIC Breakpoints

- Based on the microbiological, pharmacological (Pk/PD) and clinical data of an antimicrobial agent administered according to the **standard recommended dosage**

- Sensitive
  - ....most likely inhibit the organism in vivo.
  - High likelihood of therapeutic success

- Intermediate (indeterminate)
  - ....might inhibit the organism in vivo.
  - Uncertain therapeutic effect

- Susceptible Dose Dependent (dependent on dosing regimen)
  - ....might inhibit the organism in vivo.
  - Likelihood of therapeutic effect dependent of dosing regimen

- Resistant
  - ....will most likely not inhibit the organism in vivo.
  - High likelihood of therapeutic failure.

- Non-susceptible
  - New agents where resistant has not yet been described or when clinical correlation is still lacking for organisms displaying higher MICs
Major Pathogens of HAIs

- Emerging Multidrug-resistant Organisms (MDROs)
  - Methicillin-resistant *Staphylococcus aureus* (MRSA)
  - Vancomycin-resistant Enterococci (VRE)
  - MDR-GN
    - Carbapenem-resistant *Enterobacteriaceae* (CRE)
    - Extended Spectrum Beta-lactamases (ESBL)
    - *Acinetobacter baumannii*
    - *Pseudomonas aeruginosa*
  - *C. difficile*

Rapid Diagnostic Testing

- Molecular diagnostics—from days to hours
  - Specific pathogens
    - *C. difficile*
    - Group A Strep
  - Panels based on symptoms/sample types
    - Respiratory
    - Stool
  - Surveillance
    - MRSA
    - VRE
    - KPC
  - Resistance genes
Laboratory Information Systems

- The microbiology laboratory should choose a LIS system that can also be used by IC to data mine
- The microbiology laboratory is an “Early warning system”
  - LIS alerts
  - Statistical programs

Laboratory Information Systems

- Infection control critical values
  - Positive AFB smears and Mtb cultures
  - VRE, MRSA, GISA
  - Multi-drug resistant GNR
  - Isolation of *N. meningitidis* from sterile sites
  - Legionella

- Data summaries, monitoring trends
  - Antibiograms
  - Work Rounds between ICP and Micro
Microbiology Laboratory: Infection Control Related Functions

- Participates as a member of the infection control committee
  - provides expertise in the interpretation of culture results
  - Advice about the appropriateness and feasibility of microbiological approaches
  - Input regarding the laboratory resources necessary to accomplish the goals of the committee
  - Inform the committee of the strengths and limitations of methods employed to detect and characterize HAI pathogens

Microbiology Laboratory: Outbreak Recognition and Investigations

- Most HAIs are endemic and not associated with outbreaks
- Laboratory can alert infection control of potential outbreaks
- Laboratory must assist infection control in identifying and controlling potential outbreaks
Application of Molecular Typing Techniques

- Recognize and confirm an outbreak
  - Clusters of patients within hospitals
  - Track spread between hospitals over time
- Document hospital transmission
- Measure impact of intervention strategies
- Distinguishing relapse from re-infection in individual patients


Typing Methods

- **Phenotypic (Non-Molecular)**
  - Colony Morphology
  - Biotyping
  - Antimicrobial Susceptibility
  - Serotyping/Phage Typing

- **Molecular or Genotypic**
  - Pulsed-Field Gel Electrophoresis (PFGE)
  - Polymerase Chain Reaction
  - Arbitrarily Primed PCR
  - Antibiotic Resistance Genotyping
  - Multilocus sequence typing (MLST)
  - Single Locus sequence typing (SLST)
  - Whole Genome sequencing (WGS)
PFGE-Gold Standard
Cutting the Code

- Isolates are grown in pure culture and DNA is extracted
- Enzymes cut at specific nucleotide sequences of the DNA strand, leaving fragments of various sizes and weights behind for analysis

PFGE-Rolling the Print

- Electric current is alternated (“pulsed”) on a periodic basis
- Separates large DNA fragments called “bands” across the gel based on size
- Gels are stained and visualized under UV
- Bands are compared across isolates to determine clonality
PFGE-Matching the suspect to the scene

- Lanes 1 and 6 are “ladders” containing materials of specific weight for quality control and gel:gel comparisons.

- Lanes 2-5 are bacterial isolates; not related.

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Tenover Criteria

- Indistinguishable
- Closely related (2-3 band)
- Possibly related (4-6 band)
- Unrelated (7 or more bands)

PFGE

Advantages
- Patterns easier to interpret compared to other techniques
- Highly reproducible
- Excellent discriminatory power
- Theoretically all bacteria are typeable, some fungi as well

Disadvantages
- Cost of equipment
- Tedious
- Slow
- Certain organisms may not be typeable e.g. C. difficile, Aspergillus sp.
- May be over sensitive in detecting differences

MDR K. pneumoniae outbreak in a Cardiac Surgery Intensive Care Unit at UMMC

- 5 patients positive cultures with MDR-KP over a 15 day period in January 2010
- 8 out of 9 isolates were genetically identical

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>amikacin</td>
<td>R</td>
</tr>
<tr>
<td>ampicillin/subactam</td>
<td>R</td>
</tr>
<tr>
<td>cefazolin</td>
<td>R</td>
</tr>
<tr>
<td>cefepime</td>
<td>R</td>
</tr>
<tr>
<td>ceftriaxone</td>
<td>R</td>
</tr>
<tr>
<td>gentamicin</td>
<td>R</td>
</tr>
<tr>
<td>imipenem</td>
<td>S</td>
</tr>
<tr>
<td>piperclillin/tazobactam</td>
<td>R</td>
</tr>
<tr>
<td>SXT</td>
<td>R</td>
</tr>
<tr>
<td>gatifloxacin</td>
<td>R</td>
</tr>
<tr>
<td>polymyxin B</td>
<td>S</td>
</tr>
</tbody>
</table>

Preas et al. APIC abstract
**Klebsiella pneumoniae**

- Lanes 2-7, 9 identical
- Lanes 8 similar 2-7, 9
- Cross transmission likely

Control of the outbreak occurred after the following measures were implemented:

- Heightened attention to hand hygiene
- Enhanced environmental cleaning
- Focused disinfection of reusable patient care equipment
- Contact Precautions initiated
rep-PCR Technology

1. rep-PCR primers bind to many specific repetitive sequences interspersed throughout the genome

2. Multiple fragments of various lengths are amplified

3. Fragments can be separated by mass and charge via electrophoresis

4. A unique rep-PCR DNA fingerprint profile is created with multiple bands of varying intensity

The DiversiLab® Microbial Typing System

DiversiLab Kit with reagents and chips

DiversiLab Software
Whole Genome Sequencing in Infection Control

- 18 patients with MDR-KP at NIH-11 died
- WGS used to gain insight into why the outbreak progressed despite early IC procedures
- First paper to demonstrate that the integration of genomic and epidemiological data can yield actionable insights and control of transmission

Snitkin et al. 2012 Sci Transl Med

Epidemiologic Typing Methods
Basic Principles

- Perform only with clear objectives
- Variability exists in all methods
  - evaluate all implicated isolates simultaneously
  - compare to epidemiologically unrelated control isolates
- Demonstrate not only relatedness of clustered isolates, but differences from isolates not involved epidemiologically
Organism Storage

- Without storage supplemental tests can not be performed.
- The laboratory and infection control need to decide which isolates should be frozen and for what period of time.
- Important isolates include any isolate from a sterile site (blood, CSF, etc.), antibiotic resistant organisms (MRSA, ESBL producing isolates), and any other epidemiologically important pathogen.

Cultures of Specimens from Hospital Personnel and the Environment

- These cultures should be performed rarely and only when epidemiologically necessary.
- Detection of isolates does not determine cause.
Expanded Roles of Infection Control and Microbiology Labs

**Infection Control**
- Shift toward focused surveillance
  - ICUs
  - Devices
  - Antimicrobial resistance
- Control strategies are more proactive
  - Active intervention
  - Control of resistance

**Microbiology Labs**
- Increasingly complex and demanding work
  - Increasing resistance
  - Emerging pathogens
  - New technology
- Monitoring resistance
- Implementation of molecular epidemiology

Questions?